

CLAIMS

1. A method of treating cancer in a subject, the method comprising:
 - a) administering to a subject having cancer an effective amount of a primary cell-derived biologic comprising IL-1 β , IL-2, IL-6, IL-8, TNF- α , and IFN- γ ; and
 - b) administering to the subject an effective amount of an antagonist of programmed cell death-ligand 1 (PD-L1) or programmed cell death 1 (PD-1), wherein the administration of the primary cell-derived biologic and the administration of the antagonist occur at different locations in the subject and/or at different times.
2. The method of claim 1, wherein at least one administration of the primary cell-derived biologic occurs prior to at least one administration of the antagonist.
3. The method of claim 2, wherein at least one administration of the primary cell-derived biologic occurs prior to at least one administration of the antagonist and at least one further administration of the primary cell-derived biologic occurs after the at least one administration of the antagonist.
4. The method of claim 1, wherein at least one administration of the antagonist occurs prior to at least one administration of the primary cell-derived biologic.
5. The method of claim 4, wherein at least one administration of the antagonist occurs prior to at least one administration of the primary cell-derived biologic and at least one further administration of the antagonist occurs after the at least one administration of the primary cell-derived biologic.
6. The method of any one of claims 1 to 5, wherein the primary cell-derived biologic is administered subcutaneously or perilymphatically and the antagonist is administered intravenously or orally.
7. The method of any one of claims 1 to 6, wherein the primary cell-derived biologic is administered once a day up to 10 days and the antagonist of PD-L1 or PD-1 is administered once every two to four weeks.
8. The method of any one of claims 1 to 7, wherein the antagonist is an antisense oligonucleotide, a short interfering RNA (siRNA), small molecule, a peptide, or an antibody.
9. The method of claim 8, wherein the antagonist is an antibody.
10. The method of claim 9, wherein the antibody is a human or humanized antibody.

11. The method of claim 9 or 10, wherein the antibody is specific for PD-L1.
12. The method of claim 11, wherein the antibody is selected from the group consisting of atezolizumab, durvalumab, BMS-936559, and avelumab.
13. The method of claim 8, wherein the antagonist is CA-170.
- 5 14. The method of claim 9 or 10, wherein the antibody is specific for PD-1.
15. The method of claim 14, wherein the antibody is selected from the group consisting of nivolumab, pidilizumab, pembrolizumab, MEDI-0680, and REGN2810.
16. The method of claim 8, wherein the antagonist is AMP-224.
17. The method of any one of claims 1 to 16, wherein the subject is refractory to
10 treatment with the antagonist prior to administration of the primary cell-derived biologic.
18. The method of any one of claim 1 to 17, wherein a level of PD-L1 in a tumor of the subject increases after administration of the primary cell-derived biologic.
19. A method of treating cancer in a subject, the method comprising:
- 15 a) administering to a subject having cancer an effective amount of a primary cell-derived biologic comprising IL-1 β , IL-2, IL-6, IL-8, TNF- α , and IFN- γ ; and
b) administering to the subject an effective amount of an antagonist of programmed cell death-ligand 1 (PD-L1) or programmed cell death 1 (PD-1), wherein the antagonist is selected from the group consisting of nivolumab, pidilizumab,
20 pembrolizumab, MEDI-0680, REGN2810, AMP-224, atezolizumab, durvalumab, BMS-936559, avelumab, and CA-170.
20. The method of any one of claim 1 to 19, wherein the effective amount of the primary cell-derived biologic administered to the subject includes a ratio of IL-1 β International Units (IU) to IL-2 IU of 0.45 to 1.37, a ratio of IFN- γ IU to IL-2 IU of
25 0.19 to 0.39, a ratio of TNF- α IU to IL-2 IU of 0.53 to 1.26, a ratio of IL-6 IU to IL-2 IU of 1.16 to 6.06, and a ratio of IL-8 IU to IL-2 IU of 0.15 to 0.51.
21. The method of any one of claims 1 to 20, wherein the effective amount of the primary cell-derived biologic administered to the subject includes at least 1 IU of each of IL-1 β , IL-2, IL-6, IL-8, and TNF- α .
- 30 22. The method of any one of claims 1 to 21, wherein the effective amount of the primary cell-derived biologic administered to the subject includes 22-657 International Units (IU) or 220-6,700 pcg of IL-1 β , 29-478 IU or 1730-28,100 pcg of IL-2, 10-185 IU or

560-10,900 pcg of IFN- γ , 29-600 IU or 580-12,000 pcg of TNF- α , 34-2,895 IU or 260-22,100 pcg of IL-6 and 5-244 IU or 4,610-243,600 of IL-8.

23. The method of any one of claims 1 to 22, wherein the primary cell-derived biologic further comprises GM-CSF and G-CSF.

5 24. The method of any one of claims 1 to 23, further comprising administering to the subject a chemical inhibitor selected from the group consisting of alkylating agents, antimetabolites, antibiotics, and immunomodulating agents.

25. The method of claim 24, wherein the alkylating agent is cyclophosphamide.

10 26. The method of any one of claims 1 to 25, further comprising administering to the subject an NSAID selected from the group consisting of indomethacin, ibuprofen, celecoxib, rofecoxib, and combinations thereof.

27. The method of claim 26, wherein the NSAID is indomethacin.

28. The method of any one of claims 1 to 27, further comprising administering zinc to the subject.

15 29. The method of any one of claims 1 to 28 further comprising administering an effective amount of a cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antagonist.

20 30. The method of claim 29, wherein the CTLA-4 antagonist is an antisense oligonucleotide, a short interfering RNA (siRNA), small molecule, a peptide, or an antibody.

31. The method of claim 30, wherein the CTLA-4 antagonist is an antibody.

32. The method of claim 31, wherein the antibody is a human or humanized antibody.

33. The method of claim 32, wherein the antibody is selected from the group consisting of ipilimumab and tremelimumab.

25 34. A method of selecting a subject for treatment, the method comprising:

- a) determining a level of PD-L1 in a tumor sample obtained from a subject having cancer and to whom has been administered a primary cell-derived biologic comprising IL-1 β , IL-2, IL-6, IL-8, TNF- α , and IFN- γ ; and
 - b) administering to the subject an effective amount of an antagonist of PD-L1 or PD-1 if the level of PD-L1 in the tumor sample is higher than a threshold level of PD-L1.
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35. The method of claim 34, wherein determining comprises performing an assay to detect the level of PD-L1.

36. The method of claim 35, wherein the assay is selected from the group consisting of in situ hybridization, RT-qPCR, microarray analysis, multiplexed RNA expression analysis, RNA-seq, an immunohistochemistry assay, flow cytometry, a multiplexed protein assay and a Western blot assay.
- 5 37. The method of any one of claims 34 to 36, wherein the level of PD-L1 in the tumor sample is a level of PD-L1 in cell membranes in the tumor sample.
38. The method of claim 37, wherein determining comprises performing an immunohistochemistry assay and the threshold level of PD-L1 is partial or complete cell membrane staining in 49% of viable tumor cells in the tumor sample.
- 10 39. The method of any one of claims 34 to 38, further comprising administering the primary cell-derived biologic to the subject prior to the determining step.
40. The method of any one of claims 34 to 39, wherein the antagonist is an antisense oligonucleotide, a short interfering RNA (siRNA), small molecule, a peptide, or an antibody.
- 15 41. The method of claim 40, wherein the antagonist is an antibody.
42. The method of claim 41, wherein the antibody is a human or humanized antibody.
43. The method of claim 41 or 42, wherein the antibody is specific for PD-L1.
44. The method of claim 43, wherein the antibody is selected from the group consisting of atezolizumab, durvalumab, BMS-936559, and avelumab.
- 20 45. The method of claim 40, wherein the antagonist is CA-170.
46. The method of claim 41 or 42, wherein the antibody is specific for PD-1.
47. The method of claim 46, wherein the antibody is selected from the group consisting of nivolumab, pidilizumab, pembrolizumab, MEDI-0680, and REGN2810.
48. The method of claim 40, wherein the antagonist is AMP-224.
- 25 49. The method of any one of claims 34 to 48, wherein the effective amount of the primary cell-derived biologic administered to the subject includes a ratio of IL-1 β International Units (IU) to IL-2 IU of 0.45 to 1.37, a ratio of IFN- γ IU to IL-2 IU of 0.19 to 0.39, a ratio of TNF- α IU to IL-2 IU of 0.53 to 1.26, a ratio of IL-6 IU to IL-2 IU of 1.16 to 6.06, and a ratio of IL-8 IU to IL-2 IU of 0.15 to 0.51.
- 30 50. The method of any one of claims 34 to 49, wherein the effective amount of the primary cell-derived biologic administered to the subject includes at least 1 IU of each of IL-1 β , IL-2, IL-6, IL-8, and TNF- α .

51. The method of any one of claims 34 to 50, wherein the primary cell-derived biologic includes 22-657 International Units (IU) or 220-6,700 pcg of IL-1 β , 29-478 IU or 1730-28,100 pcg of IL-2, 10-185 IU or 560-10,900 pcg of IFN- γ , 29-600 IU or 580-12,000 pcg of TNF- α , 34-2,895 IU or 260-22,100 pcg of IL-6 and 5-244 IU or 4,610-243,600 of IL-8.
52. The method of any one of claims 34 to 51, wherein the primary cell-derived biologic further comprises GM-CSF and G-CSF.
53. A method of assessing the likelihood that a subject will be responsive to an antagonist of PD-L1 or PD-1, the method comprising:
- 10 a) administering a primary cell-derived biologic comprising IL-1 β , IL-2, IL-6, IL-8, TNF- α , and IFN- γ to a subject having a cancer that expresses a first level of PD-L1 that is below a threshold level of PD-L1; and
- b) determining a second level of PD-L1 in a tumor sample from the subject after administration of the primary cell-derived biologic, wherein a second level of PD-L1 that is above the threshold level of PD-L1 is indicative that the subject
- 15 will be responsive to the antagonist of PD-L1 or PD-1.
54. The method of claim 53, wherein determining comprises performing an assay to detect the second level of PD-L1.
55. The method of claim 54, wherein the assay is selected from the group consisting of in situ hybridization, RT-qPCR, microarray analysis, multiplexed RNA expression
- 20 analysis, RNA-seq, an immunohistochemistry assay, flow cytometry, a multiplexed protein assay and a Western blot assay.
56. The method of any one of claims 53 to 55, wherein the second level of PD-L1 is a level of PD-L1 in cell membranes in the tumor sample.
- 25 57. The method of claim 56, wherein determining comprises performing an immunohistochemistry assay and the threshold level of PD-L1 is partial or complete cell membrane staining of at least 49% of viable tumor cells in the tumor sample.
58. The method of any one of claims 1 to 57, wherein the cancer is selected from the group consisting of melanoma, lung cancer (such as non-small-cell lung cancer
- 30 (NSCLC)), renal cell carcinoma (RCC), prostate cancer, ovarian cancer, colorectal cancer (CRC), kidney cancer, gastric cancer, breast cancer, diffuse large B cell lymphoma (DLBCL), hematological malignancies (e.g., acute myelogenous leukemia (AML), multiple myeloma (MM), chronic lymphocytic leukemia (CLL), Chronic

myelogenous leukemia (CML), Hodgkin's lymphoma, non-Hodgkin's lymphoma, B-cell lymphoma, follicular lymphoma, cutaneous T-cell lymphoma), pancreatic cancer, bladder cancer, squamous cell carcinoma of the head and neck (H&NSCC also called SCCHN), genitourinary cancer, advanced cutaneous squamous cell carcinoma, liver metastases, mesothelioma, gastroesophageal cancer, Merkel cell carcinoma, and urothelial carcinoma.

59. A method of treating cancer in a subject, the method comprising:

- a) administering to a subject having cancer an effective amount of a primary cell-derived biologic comprising IL-1 β , IL-2, IL-6, IL-8, TNF- α , and IFN- γ ; and
- b) administering to the subject an effective amount of an antagonist of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), wherein the administration of the primary cell-derived biologic and the administration of the antagonist occur at different locations in the subject and/or at different times.

60. The method of claim 59, wherein at least one administration of the primary cell-derived biologic occurs prior to at least one administration of the antagonist.

61. The method of claim 60, wherein at least one administration of the primary cell-derived biologic occurs prior to at least one administration of the antagonist and at least one further administration of the primary cell-derived biologic occurs after the at least one administration of the antagonist.

62. The method of claim 61, wherein at least one administration of the antagonist occurs prior to at least one administration of the primary cell-derived biologic.

63. The method of claim 62, wherein at least one administration of the antagonist occurs prior to at least one administration of the primary cell-derived biologic and at least one further administration of the antagonist occurs after the at least one administration of the primary cell-derived biologic.

64. The method of any one of claims 59 to 63, wherein the primary cell-derived biologic is administered subcutaneously or perilymphatically and the antagonist is administered intravenously.

65. The method of any one of claims 59 to 64, wherein the primary cell-derived biologic is administered once a day up to 10 days and the antagonist is administered once every three to twelve weeks.

66. The method of any one of claims 59 to 65, wherein the antagonist is an antisense oligonucleotide, a short interfering RNA (siRNA), small molecule, a peptide, or an antibody.
67. The method of claim 66, wherein the antagonist is an antibody.
- 5 68. The method of claim 67, wherein the antibody is a human or humanized antibody.
69. The method of claim 68, wherein the antibody is selected from the group consisting of ipilimumab and tremelimumab.
70. The method of any one of claims 69 to 69, wherein the subject is refractory to treatment with the antagonist prior to administration of the primary cell-derived
10 biologic.
71. The method of any one of claim 59 to 70, wherein a level of CTLA-4 in a tumor of the subject increases after administration of the primary cell-derived biologic.
72. A method of treating cancer in a subject, the method comprising:
- a) administering to a subject having cancer an effective amount of a primary cell-
15 derived biologic comprising IL-1 β , IL-2, IL-6, IL-8, TNF- α , and IFN- γ ; and
 - b) administering to the subject an effective amount of an antagonist of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), wherein the antagonist is selected from the group consisting of ipilimumab and tremelimumab.
73. The method of any one of claim 59 to 72, wherein the effective amount of the primary
20 cell-derived biologic administered to the subject includes a ratio of IL-1 β International Units (IU) to IL-2 IU of 0.45 to 1.37, a ratio of IFN- γ IU to IL-2 IU of 0.19 to 0.39, a ratio of TNF- α IU to IL-2 IU of 0.53 to 1.26, a ratio of IL-6 IU to IL-2 IU of 1.16 to 6.06, and a ratio of IL-8 IU to IL-2 IU of 0.15 to 0.51.
74. The method of any one of claims 59 to 73, wherein the effective amount of the
25 primary cell-derived biologic administered to the subject includes at least 1 IU of each of IL-1 β , IL-2, IL-6, IL-8, and TNF- α .
75. The method of any one of claims 59 to 74, wherein the effective amount of the primary cell-derived biologic administered to the subject includes 22-657
30 International Units (IU) or 220-6,700 pcg of IL-1 β , 29-478 IU or 1730-28,100 pcg of IL-2, 10-185 IU or 560-10,900 pcg of IFN- γ , 29-600 IU or 580-12,000 pcg of TNF- α , 34-2,895 IU or 260-22,100 pcg of IL-6 and 5-244 IU or 4,610-243,600 of IL-8.
76. The method of any one of claims 59 to 75, wherein the primary cell-derived biologic further comprises GM-CSF and G-CSF.

77. The method of any one of claims 59 to 76, further comprising administering to the subject a chemical inhibitor selected from the group consisting of alkylating agents, antimetabolites, antibiotics, and immunomodulating agents.
78. The method of claim 77, wherein the alkylating agent is cyclophosphamide.
- 5 79. The method of any one of claims 59 to 78, further comprising administering to the subject an NSAID selected from the group consisting of indomethacin, ibuprofen, celecoxib, rofecoxib, and combinations thereof.
80. The method of claim 79, wherein the NSAID is indomethacin.
81. The method of any one of claims 59 to 80, further comprising administering zinc to
10 the subject.
82. The method of any one of claims 59 to 81, further comprising administering an effective amount of a PD-1 or PD-L1 antagonist.
83. The method of claim 82, wherein the PD-1 or PD-L1 antagonist is an antisense oligonucleotide, a short interfering RNA (siRNA), small molecule, a peptide, or an
15 antibody.
84. The method of claim 83, wherein the PD-1 or PD-L1 antagonist is an antibody.
85. The method of claim 84, wherein the antibody is a human or humanized antibody.
86. The method of claim 83, wherein the PD-1 or PD-L1 antagonist is selected from the group consisting of nivolumab, pidilizumab, pembrolizumab, MEDI-0680,
20 REGN2810, AMP-224, atezolizumab, durvalumab, BMS-936559, avelumab, and CA-170.
87. A method of selecting a subject for treatment, the method comprising:
- a) determining a level of CTLA-4 in a tumor sample obtained from a subject having cancer and to whom has been administered a primary cell-derived
25 biologic comprising IL-1 β , IL-2, IL-6, IL-8, TNF- α , and IFN- γ ; and
 - b) administering to the subject an effective amount of an antagonist of CTLA-4 if the level of CTLA-4 in the tumor sample is higher than a threshold level of CTLA-4.
88. The method of claim 87, wherein determining comprises performing an assay to
30 detect the level of CTLA-4.
89. The method of claim 88, wherein the assay is selected from the group consisting of in situ hybridization, RT-qPCR, microarray analysis, multiplexed RNA expression

analysis, RNA-seq, an immunohistochemistry assay, flow cytometry, a multiplexed protein assay and a Western blot assay.

90. The method of any one of claims 87 to 89, further comprising administering the primary cell-derived biologic to the subject prior to the determining step.
- 5 91. The method of any one of claims 87 to 89, wherein the antagonist is an antisense oligonucleotide, a short interfering RNA (siRNA), small molecule, a peptide, or an antibody.
92. The method of claim 91, wherein the antagonist is an antibody.
93. The method of claim 92, wherein the antibody is a human or humanized antibody.
- 10 94. The method of claim 93, wherein the antibody is selected from the group consisting of ipilimumab and tremelimumab.
95. The method of any one of claims 87 to 94, wherein the effective amount of the primary cell-derived biologic administered to the subject includes a ratio of IL-1 β International Units (IU) to IL-2 IU of 0.45 to 1.37, a ratio of IFN- γ IU to IL-2 IU of 0.19 to 0.39, a ratio of TNF- α IU to IL-2 IU of 0.53 to 1.26, a ratio of IL-6 IU to IL-2 IU of 1.16 to 6.06, and a ratio of IL-8 IU to IL-2 IU of 0.15 to 0.51.
- 15 96. The method of any one of claims 87 to 95, wherein the effective amount of the primary cell-derived biologic administered to the subject includes at least 1 IU of each of IL-1 β , IL-2, IL-6, IL-8, and TNF- α .
- 20 97. The method of any one of claims 87 to 96, wherein the primary cell-derived biologic includes 22-657 International Units (IU) or 220-6,700 pcg of IL-1 β , 29-478 IU or 1730-28,100 pcg of IL-2, 10-185 IU or 560-10,900 pcg of IFN- γ , 29-600 IU or 580-12,000 pcg of TNF- α , 34-2,895 IU or 260-22,100 pcg of IL-6 and 5-244 IU or 4,610-243,600 of IL-8.
- 25 98. The method of any one of claims 87 to 97, wherein the primary cell-derived biologic further comprises GM-CSF and G-CSF.
99. A method of assessing the likelihood that a subject will be responsive to an antagonist of CTLA-4, the method comprising:
- 30 a) administering a primary cell-derived biologic comprising IL-1 β , IL-2, IL-6, IL-8, TNF- α , and IFN- γ to a subject having a cancer that expresses a first level of CTLA-4 that is below a threshold level of CTLA-4; and
- b) determining a second level of CTLA-4 in a tumor sample from the subject after administration of the primary cell-derived biologic, wherein a second

level of CTLA-4 that is above the threshold level of CTLA-4 is indicative that the subject will be responsive to the antagonist of CTLA-4.

100. The method of claim 99, wherein determining comprises performing an assay to detect the second level of CTLA-4.

5 101. The method of claim 100, wherein the assay is selected from the group consisting of in situ hybridization, RT-qPCR, microarray analysis, multiplexed RNA expression analysis, RNA-seq, an immunohistochemistry assay, flow cytometry, a multiplexed protein assay and a Western blot assay.

10 102. The method of any one of claims 59 to 101, wherein the cancer is selected from the group consisting of melanoma, lung cancer (such as non-small-cell lung cancer (NSCLC)), renal cell carcinoma (RCC), prostate cancer, ovarian cancer, colorectal cancer (CRC), kidney cancer, gastric cancer, breast cancer, diffuse large B cell lymphoma (DLBCL), hematological malignancies (e.g., acute myelogenous leukemia (AML), multiple myeloma (MM), chronic lymphocytic leukemia (CLL),
15 Chronic myelogenous leukemia (CML), Hodgkin's lymphoma, non-Hodgkin's lymphoma, B-cell lymphoma, follicular lymphoma, cutaneous T-cell lymphoma), pancreatic cancer, bladder cancer, squamous cell carcinoma of the head and neck, genitourinary cancer, advanced cutaneous squamous cell carcinoma, liver metastases, mesothelioma, gastroesophageal cancer, Merkel cell carcinoma, and urothelial
20 carcinoma.